PREVALENCE, EMERGENCE AND FACTORS ASSOCIATED WITH A VIRAL PAPILLOMATOSIS AND CARCINOMATOSIS SYNDROME IN WILD, REINTRODUCED AND CAPTIVE WESTERN BARRED BANDICOOTS (*PERAMELES BOUGAINVILLE*)

Running head: Viral papillomstosis in *Perameles bougainville*

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Abstract: Once widespread across western and southern Australia, wild populations of the western barred bandicoot (WBB) are now only found on Bernier and Dorre Islands, Western Australia. Conservation efforts to prevent the extinction of the WBB are presently hampered by a papillomatosis and carcinomatosis syndrome identified in captive and wild bandicoots, associated with infection with the bandicoot papillomatosis carcinomatosis virus type 1 (BPCV1). This study examined the prevalence and distribution of BPCV1 and the associated syndrome in two island and four mainland (reintroduced and captive) WBB populations in Western Australia, and factors that may be associated with susceptibility to this syndrome. BPCV1 and the syndrome were found in the wild WBB population at Red Cliff on Bernier Island, and in mainland populations established from all or a proportion of founder WBBs from Red Cliff. BPCV1 and the syndrome were not found in the wild population on Dorre Island, or in the mainland population founded by animals exclusively from Dorre Island. Findings suggested that BPCV1 and the syndrome were disseminated into mainland WBB populations through the introduction of affected WBBs from Red Cliff. No difference in susceptibility to the syndrome was found between Dorre Island, Bernier Island and island-cross individuals. Severity of lesions and the number of affected animals observed in captivity was greater than that observed in wild populations. This study provided epidemiological evidence to support the pathological and molecular association between BPCV1 infection and the papillomatosis and carcinomatosis syndrome, and revealed increasing age as an additional risk factor for this disease.

Key words: BPCV1, epidemiology, papillomatosis, carcinomatosis, *Perameles bougainville*, western barred bandicoot

INTRODUCTION

The western barred bandicoot (WBB) *Perameles bougainville* was once widespread across western and southern Australia. Now extinct on the Australian mainland apart from captive and reintroduced colonies, wild populations are known only to exist on Bernier and Dorre Islands, which lie 50 kilometres west of the Western Australian coast within Shark Bay (Seebeck et al. 1990; UNESCO 1991; Short et al. 1997; Richards and Short 2003). Conservation efforts to prevent the extinction of the WBB involve captive breeding and reintroduction onto islands free of introduced predators, or into predator proof enclosures, or fox and cat baited habitat, located in the species' historic distribution range on mainland Western and South Australia. These efforts are compromised by the presence of the bandicoot papillomatosis carcinomatosis virus type 1 (BPCV1)-associated papillomatosis and carcinomatosis syndrome, observed in both captive and wild WBBs (Woolford et al. 2007; Bennett et al. 2008; Woolford et al. 2008). Previous studies have shown strong pathological and molecular evidence to support BPCV1 as the causative agent for this syndrome. This study seeks to build upon previous findings by examining epidemiological features of this syndrome in wild and captive WBB populations, and factors that may be associated with its occurrence.

The papillomatosis and carcinomatosis syndrome is characterised by multicentric proliferative lesions involving cutaneous and muco-cutaneous surfaces, seen clinically to increase in size with time (Woolford et al. 2008). Grossly and histologically the smaller epithelial lesions resemble papillomas, whereas the larger lesions are most commonly carcinoma *in situ* and squamous cell carcinomas. Involvement of the feet, eyes and the mouth, leads to problems with ambulation, vision and eating, and affected animals may die due to secondary infections or are euthanased on welfare grounds. Awareness of this syndrome first arose in 1999, when lesions were noticed developing on

a captive adult female housed at Kanyana Wildlife Rehabilitation Center in Perth, Western Australia (Thomas and Friend 2003). Within a year, additional WBBs housed at the facility were noted to have developed lesions. In 2000, similar disease was noticed in captive WBBs at another breeding colony north of Perth in Shark Bay, and identified on wild WBBs on Bernier Island by the Western Australian Department of Environment and Conservation (DEC) in 2001. A historical trace back performed on 93 museum specimens from five museums in Australia found similar lesions to be present on three Western Australian Museum WBB specimens from Red Cliff on Bernier Island collected in 1982 (n = 2) and 1988 (n = 1) (Hill 2003; Hill 2005).

Bernier and Dorre Islands are of vital importance in the preservation of the only remaining wild populations of the WBB, along with other Australian marsupials also classified as rare and/or endangered (Short et al. 1997). White Beach on Dorre Island and Red Cliff on Bernier Island have been the focus of regular fauna monitoring activities since 1987, and these sites were used for collection of WBBs used to establish mainland populations in Western and South Australia. Skin abnormalities were not recorded by researchers examining these wild populations prior to the emergence of the papillomatosis and carcinomatosis syndrome in captive populations.

The purpose of this study is to examine the epidemiological association between BPCV1 and the papillomatosis and carcinomatosis syndrome in multiple, geographically isolated, wild, reintroduced and captive WBB populations in Western Australia. Furthermore, a retrospective study was conducted to investigate the pattern of disease emergence in mainland WBB populations, and examine variables that may be associated with altered susceptibility to this syndrome.

METHODS

Cross-sectional study populations and sampling technique

Remnant wild populations of the WBB exist on Bernier and Dorre Islands, Western Australia (25°00'S, 113°07'E; Figure 1). The most recent estimated WBB population on the islands, carried out between 2006 and 2008, inclusive, is 445 (95% confidence interval 268-704) on Dorre Island, and 286 (95% CI 129-495) on Bernier Island (L. Reinhold pers. comm., unpublished government report). Prior to this survery, the combined population for the islands had been estimated to lie between 2200-4400, however this range was considered subject to substantial and rainfall dependent climatic fluctuations (Short et al. 1997). Reintroduced mainland populations of WBBs founded by individuals from Bernier and/or Dorre Islands exist in predator-proof, extensive range enclosures at Heirisson Prong (Dorre Island founder WBBs), Shark Bay, Western Australia (26°04'S, 113°22'E)(Richards and Short 2003); the Dryandra Field Breeding Center (DFBC, Bernier and Dorre Island founder WBBs), Western Australia (32°45'S, 116°55'E) (Friend and Beecham 2004); Faure Island (Heirisson Prong founder WBBs), Shark Bay, Western Australia (25°51'S, 113°53'E) (Richards 2006); and at the Arid Recovery Project (ARP, Bernier Island founder WBBs) reserve in northern South Australia (30°31'S, 136°53'E) (Moseby and Read 2006) (Figure 1). The Faure Island and ARP populations were not monitored by the authors during the course of this study.

Prior to the cessation of captive breeding activities in 2005, captive populations existed at the Peron Captive Breeding Center (PCBC), Shark Bay, Western Australia (PCBC 25°50'S, 113°53'E) and at the Kanyana Wildlife Rehabilitation Center (KWRC) Perth, Western Australia (31°57'S, 116°03'E) (Figure 1). The PCBC population was founded by WBBs exclusively from Bernier Island (Morris et al. 2004), and the KWRC population founded by individuals from both Bernier and Dorre Islands (Butcher 2005).

Wild and reintroduced WBB populations were monitored by the authors periodically between 2000 and 2007 inclusive (Table 1). WBBs were trapped overnight using Elliot and Sheffield cage traps set within the species' preferred habitat [coastal sandplain heath and hummock grassland, dunal systems (heath and scrub) and travertine heath (Short et al. 1998)]. Sampling sites were chosen based on WBB preferred habitat and accessibility, sampling was opportunistic and sample sizes determined by trapping success and number of nights spent at each location. All trapped individuals were sampled. Prevalence of the papillomatosis and carcinomatosis syndrome was calculated as the [individuals affected by syndrome]/ [individuals in the population examined] during each trapping exercise. Captive populations of the WBB (PCBC and KWRC) were monitored by center staff and the authors from the time of inception until the cessation of captive breeding activities due to the presence of the papillomatosis and carcinomatosis syndrome. Remaining animals from each colony were quarantined in isolation at the KWRC from February 2005 and no further captive breeding activities were conducted.

The case definition for the papillomatous and carcinomatous syndrome is the presence of solitary or multicentric proliferative lesions of the skin and mucosal surfaces; histologically, these lesions resemblepapillomas, carcinoma *in situ*, or carcinomas of squamous epithelia.WBBs were examined under mask delivered isoflurane anaesthesia. Swabs were taken from epithelial surfaces of both clinically normal WBBs and those with evidence of epithelial abnormalities using sterile 0.9% NaCl soaked cotton-tip swabs for the purpose of viral DNA detection (Martens et al. 2001; Antonsson and Hansson 2002; Woolford et al. 2007). Sites swabbed comprised skin of the lateral flank, lip commissure and feet and the conjunctival epithelia (sites at which epithelial lesions are most commonly observed). Abnormal (e.g. alopecic, thickened, inflamed, eroded or ulcerated) mucosal or cutaneous sites were also swabbed. Following collection in the field, swabs were stored at -20°C until processed in the laboratory and tested by PCR techniques for the detection of BPCV1 DNA as

described previously (Woolford et al. 2007). When appropriate, surgical biopsies were collected from epithelial lesions of affected WBBs under isoflurane anaesthesia. Whole DNA was extracted from biopsy tissues and subjected to PCR techniques for the detection of BPCV1 DNA as described previously (Woolford et al. 2007). Archived formalin-fixed paraffin-embedded tissue from affected WBBs was screened using *in situ* hybridisation (ISH) targeting BPCV1 genomic sequences as previously described (Bennett et al. 2008).

Retrospective study (captive) populations and statistical analyses

A retrospective study was conducted examining records from individuals bred and/ or held at KWRC between 1994 and 2005, inclusive (n = 57), and at the PCBC between 1998 and 2005, inclusive (n = 70). Individuals who died at 6 months of age or younger were excluded from the study (youngest recorded WBB affected by the papillomatosis and carcinomatosis syndrome ~10 months of age).

Records pertaining to observational studies, husbandry, veterinary procedures and diagnostic procedures were sourced from the Western Australian Department of Environment and Conservation, KWRC, Dr Jeff Short (Heirisson Prong), the Adelaide and Monarto Zoological Gardens, VETPATH Laboratory Services (VLS), the Western Australian Department of Agriculture and Food (DAFWA), IDEXX Diagnostic Veterinary Pathology and the conservation medicine and pathology sections at the Murdoch University School of Veterinary and Biomedical Sciences. Microsoft Access™ 2003 and Excel® 2003 databases (Microsoft Corporation, Washington, USA) were utilised to collate and analyse data.

Statistical analyses examining the relationship between disease status and categorical variables (genetic origin, sex, disease status of sire and dam) were performed using Chi-Square and Fisher's exact tests in SPSS 15.0 for Windows® (SPSS Inc., Chicago, Illinois). Odds ratios (ORs) were used for the analysis of data when significant differences between groups were observed. OR 95% confidence intervals were calculated using Woolf's method, $e^{(\ln(\text{odds ratio}) \pm 1.96 \sqrt{(\text{Variance of } \ln(\text{OR}))})}$ (Kahn and Sempos 1989). T-tests used to examine differences between continuous data sets (longevity, age at which disease first detected) were also performed using SPSS 15.0 for Windows® (SPSS Inc., Chicago, Illinois). Distribution of continuous data was inspected for normality through the generation of histograms and calculation of skewness and kurtosis values. Levene's test was performed to examine for homogeneity of variance between groups (when applicable).

Evaluation of age as a risk factor for disease was performed using life tables constructed on the probability of being affected by, or remaining unaffected by, the syndrome with increasing age (grouped at six monthly intervals). Calculations were performed using age range (x), number alive at start of period (n_x), number that died during time period i.e. withdrawn (w_x), the remnant at-risk population (r_x; $r_x = n_x -1/2\ w_x$), new cases of the syndrome during time period (d_x), probability of developing the syndrome at age range x (q_x; $q_x = d_x/r_x$), probability of not developing the syndrome at age range x (p_x; $p_x = 1-q_x$), and the cumulative probability of remaining free from the syndrome at x years of age (P_x; $P_x = p_x P_x-1$). Survival curves were constructed examining P_x against age grouping.

The minimum sample size (n) required to detect disease (assuming a pefect test) in the wild populations examined, was calculated using a formula based on hypergeometric sampling, $n = [1-(\alpha)^{1/D}][N - (D - 1)/2]$, where N = population size, $\alpha = 1 -$ confidence, and D = expected number of diseased animals in the population.

8

RESULTS

Prevalence of the BPCV1-associated papillomatosis and carcinomatosis syndrome in wild, reintroduced and captive WBB populations

Wild and reintroduced populations

Detection and prevalence of the BPCV1-associated papillomatosis and carcinomatosis syndrome in wild and reintroduced mainland populations of the WBB is presented in Table 1. Between 2000 and 2007, BPCV1 and clinical evidence of the papillomatosis and carcinomatosis syndrome were detected in WBBs examined at Red Cliff on Bernier Island (n = 9/ 51) and at the Dryandra Field Breeding Center (DFBC) (n = 10/ 107). BPCV1 was detectable only in individuals affected by the papillomatosis and carcinomatosis syndrome, and BPCV1 DNA was amplifiable from lesions of 12/14 WBBs whom lesions were screening by PCR or ISH. Viral DNA was not amplifiable from 2 affected individuals, for whom only formalin-fixed paraffin-embedded archival tissues were available for testing. BPCV1 DNA was not amplifiable from skin and mucosal swabs taken from 24 clinically normal WBBs on Bernier Island, nor from 13 clinically normal WBBs at the DFBC.

Clinical evidence of the papillomatosis and carcinomatosis syndrome was not detected in WBB populations on Dorre Island (n = 74) or at Heirisson Prong (n = 66). BPCV1 DNA was not amplfiable from skin and mucosal swabs taken from 21 clinically normal WBBs on Dorre Island, and 11 clinically normal WBBs on Heirisson Prong. Calculated sample sizes required to detect disease on Dorre Island at assumed prevelances of either 5%, or 10% (equivalent to minimum disease prevelance observed in affected populations in this study) with 95% confidence were 55 and 28 individuals, respectively. In total, 74 individuals were examined on Dorre Island, which was

greater than the number required to demonstrate freedom from disease if calculated using the above parameters.

Captive populations

Of the 57 WBBs housed at the KWRC for which records were available, 30 developed clinical evidence of the papillomatosis and carcinomatosis syndrome (Table 2). Histopathological evaluation of lesions in 21 individuals confirmed the syndrome in each, and BPCV1 DNA was detected by PCR and/ or ISH. BPCV1 DNA in skin lesions from all of 10 WBBs screened by these methods. Of the 70 WBBs housed at the PCBC for which records were available, 43 developed clinical evidence of the papillomatosis and carcinomatosis syndrome. Histopathological evaluation of lesions in 20 individuals confirmed the syndrome in each, and BPCV1 DNA was detected by PCR and/ or ISH BPCV1 DNA in skin lesions from 12/16 WBBs screened by these methods.Viral DNA was not amplifiable from 4 affected individuals, for whom only formalin-fixed paraffin-embedded archival tissues were available for testing. The severity of lesions (Woolford L., unpublished data) and the number of affected animals as determined by period prevelance was greater in captivity than observed in wild populations (Table 1).

Emergence of the papillomatosis and carcinomatosis syndrome in wild, reintroduced and captive WBB populations

The translocation history of wild caught and captive bred individuals and the pattern of disease emergence in mainland populations are presented in Figure 2. The papillomatosis and carcinomatosis syndrome and BPCV1 were found to be present in the Bernier Island WBB population at Red Cliff and in captive populations comprising all or a proportion of founder WBBs

from Red Cliff (KWRC, DFBC and the PCBC). Neither BPCV1 nor clinical evidence of the

papillomatosis and carcinomatosis syndrome were observed in WBBs examined from the Dorre

Island or Heirisson Prong (Dorre Island derived founder stock only) populations. In these

geographically isolated populations studied, the syndrome did not exist independently of the

presence of the virus (and vice-versa). The disease and BPCV1 status of wild, reintroduced and

captive WBB populations at the end of the study period (2007) is presented in Table 2.

Evaluation of risk or susceptibility to BPCV1 infection and the papillomatosis and carcinomatosis

syndrome

Analyses were performed using pooled records of affected and unaffected WBBs from the PCBC (n

= 70) and KWRC (n = 57) to investigate factors common to both populations. Of the 127 WBBs for

which complete records were available, 73 WBBs (57.5%) developed the papillomatosis and

carcinomatosis syndrome. The mean lifespan of WBBs affected by the papillomatosis syndrome,

when known, (n = 57, mean = 4y3m, std. dev. = 1.5) was found to be significantly higher in

affected WBBs when compared with unaffected individuals (n = 32, mean = 2y9m, std. dev. = 1.50;

t(89) = -4.46, p < 0.000). The magnitude of difference between the unaffected and affected groups

was found to be large (eta squared = 0.19). Causes of death in affected animals, known for 34/73

WBBs, were euthanasia due to severe skin disease (n = 25), presumed natural death/ old age (n = 5),

haemorrhagic gastroenteritis (n = 2), fighting (n = 1) and following manual restraint (n = 1). Causes

of death in unaffected animals, known for 11/ 54 WBBs, were presumed natural death/ old age (n =

3), fighting (n = 1), infection post eye ablation (n = 1), manual restraint during fitting of radio collar

(n = 2) and toxoplasmosis (n = 4). The mean age at which skin lesions were detected (when known,

n = 44) in affected WBBs bred or held at a captive breeding facility was 3 years 2 months (range

10m – 6y6m, std. dev. = 1.3).

In captive individuals affected by the syndrome, both lifespan and age at diagnosis were known for

44/73 individuals, and in unaffected animals, the lifespan was known for 32/ 55 individuals. These

76 animals were matched for age and the number of new cases recorded and the proportion of

individuals affected were determined for each age group (grouped at six monthly intervals).

Constructed life table and survival curve (Table 2 and Figure 3) indicated that the cumulative

probability (P_x) of remaining free from the papillomatosis and carcinomatosis syndrome decreased

with increasing age, i.e. the risk of developing this disease increased as the individual aged. The

greatest incidence of new cases occured in individuals greater than 2 years of age (Table 2).

Transmissibility of the papillomatosis and carcinomatosis syndrome from dam to pouch young was

investigated by comparing the frequency of the syndrome in WBBs whose dam developed the

syndrome and those whose dam did not. Univariant analysis revealed no significant difference

between the two groups (Fisher's exact test, p = 0.422). No breeding females were recorded as

exhibiting clinical disease whilst carrying pouch young. In individuals whose dam developed the

syndrome, no significant difference in risk was observed between individuals whose dam developed

warts less than 2 years after the individual was born vs. those whose dam developed warts two years

or more after the individual was born (Fisher's exact test, p = 0.72).

The Kanyana captive colony, comprising WBBs from both geographically isolated islands and

island-cross ('hybrid') individuals, was examined for genetic susceptibility to developing the

papillomatosis and carcinomatosis syndrome. Univariant analyses performed with 57 individuals of

known ancestry (30 affected and 27 unaffected) revealed no significant difference in susceptibility

to developing the syndrome on the basis of genetic origin (Bernier vs. Dorre vs. island-cross) (n = 57, Chi-Square test, p = 0.709): (n = 33, Chi-Square test, p = 0.955). Univariant analyses performed with data from both colonies revealed no significant difference in disease susceptibility based on whether the individual's dam and/ or sire developed skin disease or between the sexes.

DISCUSSION

Evaluation of risk or susceptibility to BPCV1 infection and the papillomatosis and carcinomatosis syndrome

In wild, reintroduced and captive populations examined, BPCV1 was consistently and reliably detected in association with lesions of individuals clinically affected by the papillomatosis and carcinomatosis syndrome, and not at all in clinically normal individuals. This disease was found to affect adult WBBs, and in captive populations, the probability of developing the syndrome increased with age. This increased probability may be due to greater opportunity for viral transmission, a long pre-lesional viral incubation period, or long periods of latent viral infection. Alternatively, endogenous or exogenous factors may be introduced as a WBB ages that increase susceptibility to BPCV1 infection, or trigger the development of clinical disease following a period of viral latency. Activation of infections with the similar papilloma and polyomaviruses, can occur through immunosuppression of the host (Benton et al. 1992; Campo et al. 1994) or through mechanical trauma (Siegsmund et al. 1991). The mean longevity in affected WBBs was found to be significantly higher than their unaffected counterparts. Furthermore, the average longevity of unaffected WBBs in this study was less than the mean age of lesion detection in affected WBBs, prompting one to consider that if these unaffected WBBs had lived longer they too may have developed the syndrome.

In this study we considered whether the BPCV1 papillomatosis and carcinomatosis syndrome may be an expression of an underlying and more consequential immunological defect in the WBB. A recent study by Smith and Hughes (2007a) found genetic diversity levels in the WBB, estimated by genotyping six microsatellite loci across 134 individuals from 5 populations (Bernier and Dorre Islands, Heirisson Prong, the PCBC and Dryandra), to be amongst the lowest ever recorded for a population of marsupials (Smith and Hughes 2007a). Reduced genetic diversity may increase susceptibility of a species to novel diseases, environmental stresses and, ultimately extinction (World Conservation Monitoring Centre 1992; Frankham 1997; Smith and Hughes 2007a). An increased incidence of similar, papillomavirus-associated disease has also been reported in other endangered species (Joslin et al. 2000; Bossart et al. 2002; Tachezy et al. 2002b).

The severity of lesions and the number of affected animals observed in captivity was greater than that observed in wild populations. We considered that these findings may be biased by the observation of individuals in captive breeding facilities for the greater part of their natural lives, in contrast to wild caught individuals in which almost all were examined once only. WBBs in captivity may live up to 8 years of age, therefore falling into higher risk age groups, whereas in the wild individuals may only live to 4 years of age or less (N. Thomas pers. comm. 2005; J. Butcher pers. comm. 2005; J. Short pers. comm. 2006). The greater prevalence, and perhaps severity, of disease in captivity may occur because WBBs are living longer, with death in wild populations occurring earlier from risks not present in captivity, e.g. predation, nutritional and environmental stressors. In addition, affected wild WBBs may be more susceptible to predation or death from other stressors, therefore less frequently encountered. WBBs are territorial and solitary animals, therefore it should be considered whether captivity promotes unnaturally long and frequent contact between individuals, facilitating transmission of agents such as BPCV1.

Prevalence of the BPCV1 and the papillomatosis and carcinomatosis syndrome in wild, reintroduced and captive WBB populations

The BPCV1-associated papillomatosis and carcinomatosis syndrome was found to be present in the Bernier Island WBB population at Red Cliff and in all reintroduced populations comprising founder WBBs from Red Cliff (DFBC, KWRC, and PCBC). BPCV1 was not detected in, and clinical evidence of the papillomatosis and carcinomatosis syndrome was not observed in, WBBs examined from the Dorre Island or Heirisson Prong populations. Although BPCV1 and the syndrome have not yet been detected on Dorre Island, this study found no statistically significant difference in susceptibility to the syndrome between Dorre Island, Bernier Island and island-cross populations.

In attempting to explain the pattern of disease emergence in WBB populations, the authors considered that BPCV1 was present in the Red Cliff WBB population only, and subsequently disseminated in mainland populations via the introduction of infected animals from Red Cliff. Findings from a recent study suggest that BPCV1 has been present in the WBB population for at least 10 million years (Bennett et al. 2008). Consequently, the papillomatosis and carcinomatosis syndrome may have been present in Bernier Island WBBs for equally as long, and went undetected, or was thought to be innocuous, by researchers prior to 2001. This and previous studies have failed to find evidence of affected individuals in the Dorre Island and Heirisson Prong populations. The apparent, and serendipitous, freedom of these populations from disease is curious, and reasons for which remain obscure. One proposed explainantion for the isolation of BPCV1 in the Bernier Island WBB population is that it occurred through a combination of rising sea levels, leading to the separation of the islands 3000 years ago (Hancock et al. 2000), and a substantial population decline on Dorre Island, leading to the loss of BPCV1 infected animals, and hence BPCV1. Further research into this area is needed to further explain this finding.

The Dorre Island sample size in this study was greater than the calculated minimum required to detect disease if it occurred a similar or lesser prevelance to disease on Bernier Island, inferring it unlikely that disease on Dorre went unnoticed due to insufficient sample sizes. However, affected animals may have been missed if they were resident in areas not included in sampling sites. During this study, sample sizes were small and limited sites sampled on each island due to time, logistical and financial restrictions. Chosen sites supported WBB preferred habitat and were safe for boat landings (non-random/ convenience sampling). Due to this bias, the cohort may not be representative of nor be reliably extrapolated to reflect the entire WBB population on each island. However, if these areas are to remain collection sites for mainland translocations, it is important that they are prioritised in population health monitoring exercises.

An additional limitation of this study was its inability to detect pre-lesional, carrier state or latently infected individuals using the methodolgies described. Based on the knowledge of similar virus familes (Papilloma- and Polyomaviridae), it is possible that BPCV1 virions may be sequested within the skin or adnexae, and infection reactivated following epithelial trauma or immunosuppression. Only clinically evident lesions tested positive for BPCV1 DNA using the skin swab technique, and it appears that detection of BPCV1 DNA by this method requires a lesion which is shedding virions or viral DNA in the superficial epithelium. Seroconversion in BPCV1-infected WBBs has not yet been investigatied, but may be an unreliable indicator of exposure given the apparently exclusively epitheliotropic BPCV1 lifecycle.

Emergence of the papillomatosis and carcinomatosis syndrome in wild and mainland WBB populations

The BPCV1-associated papillomatosis and carcinomatosis syndrome observed to emerge in mainland WBB populations in recent times appears to have originated on Bernier Island at Red Cliff and disseminated into mainland captive breeding colonies at PCBC, KWRC and DFBC through the introduction of affected WBBs from Red Cliff. Due to its similarity to other small DNA viruses such as papillomaviruses and polyomaviruses, BPCV1 is likely an ancient and species-specific virus that has co-evolved with its WBB host (Van Ranst et al. 1995; Crandall et al. 2006; Perez-Losada et al. 2006; Rector et al. 2007). A second, highly similar but distinctly different virus, BPCV2, was recently detected in papillomatous skin lesions from a mainland southern brown bandicoot (*Isoodon obesulus*) in Western Australia (Bennett et al. 2008). The evolutionary divergence of BPCV1 and BPCV2 is proposed to have occurred approximately 10.2 million years ago, coincident with the divergence of the two extant genera within the family Peramelidae, *Isoodon* and *Perameles*.(Nilsson et al. 2004; Bennett et al. 2008) This finding is both supportive of the theory of host-virus co-evolution and suggestive that BPCV1 has been present in the WBB population for at least 10 million years (Bennett et al. 2008). Consequently, the associated papillomatosis and carcinomatosis syndrome may have been present in Bernier Island WBBs for equally as long, and went undetected, or was thought to be innocuous, by researchers prior to 2000. Alternatively, this syndrome may have become apparent in recent times due to a change in its clinical expression, perhaps triggered by the introduction of external factors, such as environmental changes, infection with other agents, or changes in immune function. Although not conforming to current knowledge of the behaviour of papilloma- and polyomaviruses, transmission of BPCV1, or a related virus, from another species to the WBB cannot be ruled out.

Impact of the papillomatosis and carcinomatosis syndrome on conservation efforts

Clinical implications for individuals affected by the papillomatosis and carcinomatosis syndrome are certainly unpleasant and debilitating in the advanced stages of disease, and a considerable proportion of WBBs held in captive breeding facilities developed the syndrome. A quantitative measure of impact of this syndrome on the fecundity, recruitment, survival, and hence size, of wild populations was not able to be determined due to the low numbers of animals and few sites examined on each island. Although it appears likely that BPCV1 has co-evolved with the WBB population, it is a disease of welfare significance in captive WBB populations and may also be of significance in wild populations. Furthermore, the potential effect of increasing external stressors, such as environmental, climatic, nutritional or disease factors, on the expression of this syndrome in wild populations, remains unknown.

These findings have been communicated to the Western Australian Department of Environment and Conservation and the Recovery Team responsible for WBB conservation and recovery. One key recommendation presented to these agencies was establishment of more disease-free reintroduction and captive breeding populations of WBBs. From the perspective of minimising or eliminating the papillomatosis and carcinomatosis syndrome in the future, the absence of observable clinical disease and the failure to detect BPCV1 in Dorre Island WBBs makes this population the best source for captive breeding and mainland reintroductions. Due to the presence of BPCV1 and the papillomatosis and carcinomatosis syndrome at Red Cliff, further use of this population will likely contribute to the persistence of the syndrome in mainland populations. However, an important argument exists for maintaining the use of the Bernier Island WBBs. Island populations display less genetic variation than their mainland counterparts and are also more susceptible to extinction (World Conservation Monitoring Centre 1992; Frankham 1997). Genetic factors believed to contribute higher extinction rates include inbreeding depression, loss of genetic variation, accumulation of mildly deleterious mutations, and genetic adaptations to island environments

(limited ability to avoid predators and diseases) (Frankham 1997). Smith and Hughes (2007a) found that the contemporary isolation of Bernier and Dorre Islands has acted to maintain a higher combined effective WBB population size than would be expected from either population on its own, and that future use of both genetic reservoirs would act to maximise genetic diversity for mainland reintroductions (Smith and Hughes 2007a). Future efforts to insure against threats to the survival and well-being of WBBs require that a greater number of geographically separate colonies of WBBs be established within predator-proof enclosures. To maximize the genetic diversity within these colonies, it has been recommended that intensively managed captive breeding programs be reinstated, using carefully selected BPCV1-free WBBs descended from both Bernier Island and Dorre Island stock. Program recommendations include viral screening during suitably long quarantine periods, managed captive breeding of disease free individuals only, and regular and thorough monitoring of established colonies. These measures are of course fallible, and the risk that BPCV1-infected individuals will enter mainland populations can only be minimised, not eliminated.

CONCLUSIONS

This study is the first to document the emergence, geographical distribution and factors associated with the papillomatosis and carcinomatosis syndrome in the western barred bandicoot. This recently emerged syndrome appears to have originated at Red Cliff on Bernier Island and subsequently disseminated into the mainland WBB populations through the introduction of affected WBBs from this site. In these geographically isolated populations, the syndrome did not exist independently of the presence of BPCV1 (and vice-versa). Both the clinical syndrome and BPCV1 were found to be present in the population at Red Cliff, and in all captive populations comprising all or a proportion of founder WBBs from Red Cliff (Kanyana, Dryandra and the PCBC). Neither BPCV1 nor clinical evidence of the syndrome were observed in WBBs from the Dorre Island or Heirisson Prong (Dorre

Island derived) populations. These findings support strong molecular (Woolford et al. 2007; Bennett et al. 2008) and pathological (Woolford et al. 2008) evidence for the existence of a causal relationship between BPCV1 and the papillomatosis and carcinomatosis syndrome. This syndrome affects adult WBBs, and the probability of developing the syndrome increases with age. Whilst the impact of this disease in wild WBB populations was not determined compared with captive populations, it remains a considerable welfare issue in breeding facilities and affected animals are highly unsuitable for reintroduction programmes. Furthermore, the potential effect of external stressors, such as environmental, climatic or nutritional factors, on the expression of this syndrome in wild populations, remains unknown. Further research is required to improve knowledge of this syndrome, one of many hurdles in the recovery of this endangered marsupial.

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Table 1 Disease screening of wild, reintroduced and captive WBBs populations 2000-2007, inclusive.

Population		Source of founders	*t* period	N° WBB	Disease +ve	Period prevalence	Cases confirmed by histology	BPCV1 +ve? (PCR)	Most recent colony status
Bernier Island	Red Cliff	n/a	2000 – 2007	51	9	17.6% (Range 10.5-41.7%)	3	Affected = 4/4 Unaffected = 0/18	**Disease +ve BPCV1 +ve**
	Hospital Bay	n/a	2000 – 2007	8	0	0.0%	n/a	Unaffected = 0/6	Disease & BPCV1 free
Dorre Island	White Beach	n/a	2001 - 2007	58	0	0.0%	n/a	Unaffected = 0/21	Disease & BPCV1 free
	Disaster Cove	n/a	2003 - 2007	14	0	0.0%	n/a	Unaffected = 0/5	Disease & BPCV1 free
	Castle Point	n/a	2001	2	0	0.0%	n/a	ND	Disease & BPCV1 free
Heirisson Prong		White Beach (DI)	2005 - 2006	66	0	0.0%	n/a	Unaffected = 0/11	Disease & BPCV1 free
Dryandra Field Breeding Center		Red Cliff (BI) & White Beach (DI)	2000-2006	107	10	9.4% (Range 10.0-25.0%)	5	Affected = 8/10 Unaffected = 0/13	**Disease +ve BPCV1 +ve**
Kanyana Wildlife Rehabilitation Center		Red Cliff (BI) & White Beach (DI)	1994-2005	57	30	52.6%	21/21	Affected 10/10	**Disease +ve BPCV1 +ve Quarantine facility 2005 →**
Peron Captive Breeding Center		Red Cliff (BI)	1998 - 2005	70	43	61.4%			**Disease +ve BPCV1 +ve Closed 2005**

ND = Not Done, PCR testing of skin swabs not available in animals examined pre-May 2006; BI = Bernier Island; DI = Dorre Island

Table 2. New cases of disease in captive WBBs as grouped by six-monthly age intervals.

x	nx	wx	rx	dx	qx	px	Px
Age range	No at start of t period	Withdrawrals	At risk (nx-1/2wx)	New cases	Probability of developing disease	Probability of remaining disease free	Cummulative prob of remaining disease free
0.5 - 1	76	5	73.5	2	0.027	0.973	0.973
1 - 1.5	69	4	67	4	0.060	0.940	0.915
1.5 - 2	61	7	61.5	3	0.049	0.951	0.870
2 - 2.5	51	2	51	7	0.137	0.863	0.751
2.5 - 3	42	2	42	8	0.190	0.810	0.608
3 - 3.5	32	3	32	8	0.250	0.750	0.456
3.5 - 4	21	3	21	3	0.143	0.857	0.391
4 - 4.5	15	2	15	2	0.133	0.867	0.339
4.5 - 5	11	2	11	5	0.455	0.545	0.185
5 - 5.5	4	1	4	0	0.000	1.000	0.185
5.5 - 6	3	0	3	1	0.333	0.667	0.123
6 - 6.5	2	1	2	1	0.500	0.500	0.062

Figure 1. Distribution of past and present (2009) populations of the western barred bandicoot. Arrows indicate Bernier and Dorre Islands, sites of remnant wild populations. Captive breeding and wild-reintroduced colonies are indicated as 1. Faure Island, 2. Heirisson Prong, 3. Kanyana Wildlife Rehabilitation Centre, 4. Dryandra Field Breeding Center, and 5. the Arid Recovery Project (adapted from (Richards 2005)).
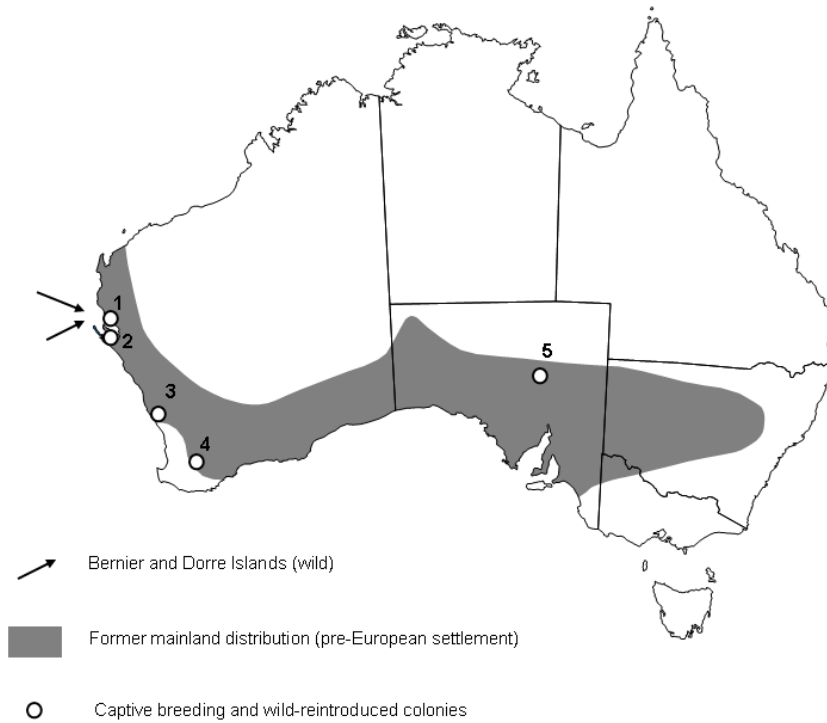
Figure 2 Sources of reintroduced and captive WBB populations, and pattern of emergence/ detection of the papillomatosis and carcinomatosis syndrome. Red highlighting indicates presence of disease.
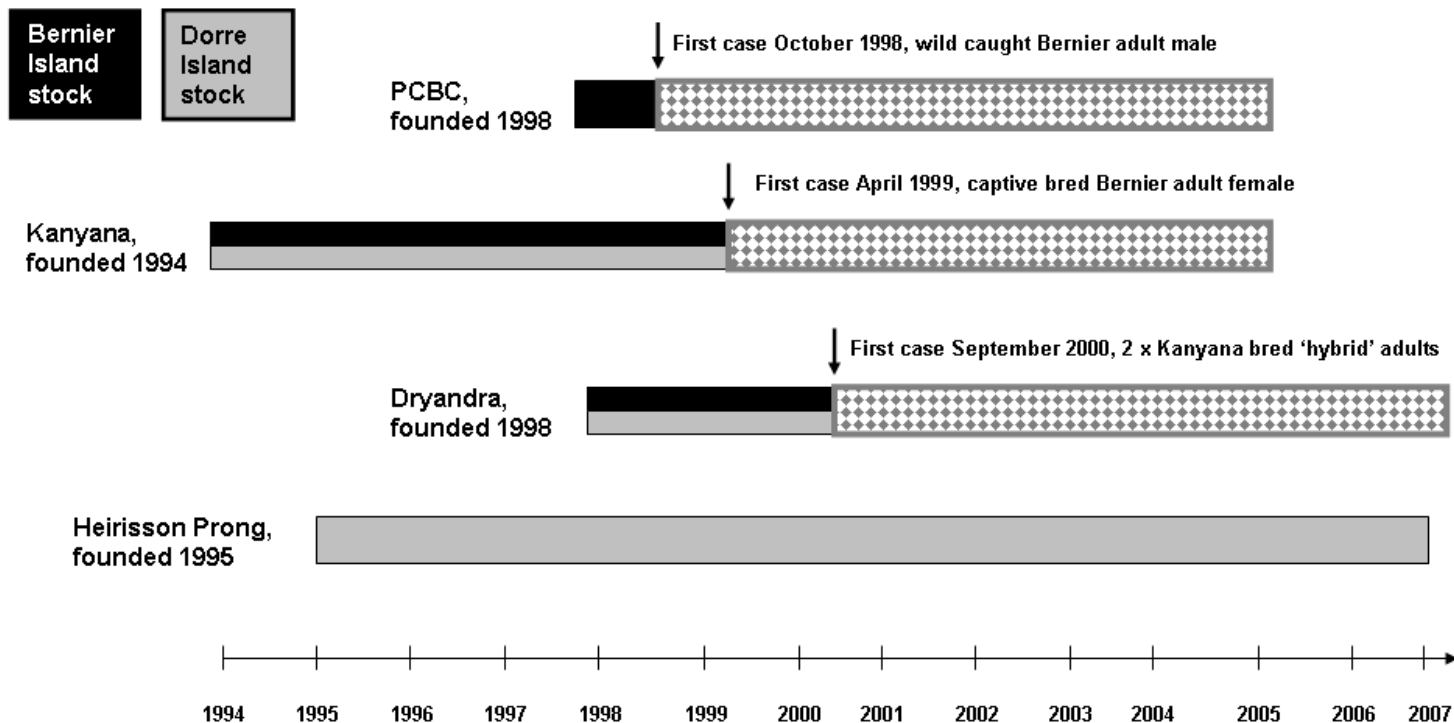
Figure 3 Cummalative probability of remaining free of the papillomatosis and carcinomatosis syndrome with progressive age. The probability of remaining disease free decreased with age, indicating that the risk of developing this disease increased as the individual aged.